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Investigation into the Synthesis of Bioactive Butenolides

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Appendix C

RESEARCH / CREATIVE PROJECT ABSTRACT / EXECUTIVE SUMMARY
FINAL REPORT FORM

Title of Project
Investigation into the Synthesis of Bioactive Butenolide

Student Name Neha Amatya

Faculty Sponsor Dr. Sara Hein

Department Chemistry

Abstract
Previous research done on coprophilous fungi has revealed the presence of secondary metabolites containing butenolides sub-structures. These butenolides show activity against the yeast Candida albicans. In efforts to further these investigations, the synthesis of butenolide analogs was undertaken. The starting materials, 2-methylacrylic acid and 1-penten-3-ol, were esterified in the presence of triethyl Iamine to form an ester, pent-1-en-3-yl-2-methylprop-2-enoate. The formation of ester was confirmed using 13C- NMR. The ester was then subjected to ring-closing metathesis in the presence of Grubb' s catalyst. The formation of the butenolide was also confirmed using 13C-NMR. The product, 5-ethyl-3-methyl-2-(5H)-furanone obtained from this step has been purified using column chromatography. The butenolide bioassay was done to test its activity against Candida.

The end product of this project in electronic format has been submitted to the Provost/Vice President for Academic Affairs via the Office of Grants & Sponsored Projects Officer (Maxwell 161, npeterson@winona.edu).

Student Signature _____ Date 5/1/2013.

Faculty Sponsor Signature _____ Date 5/1/13

Investigation into the Synthesis of Bioactive Butenolides

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Winona State University, Winona, MN 55987

Background

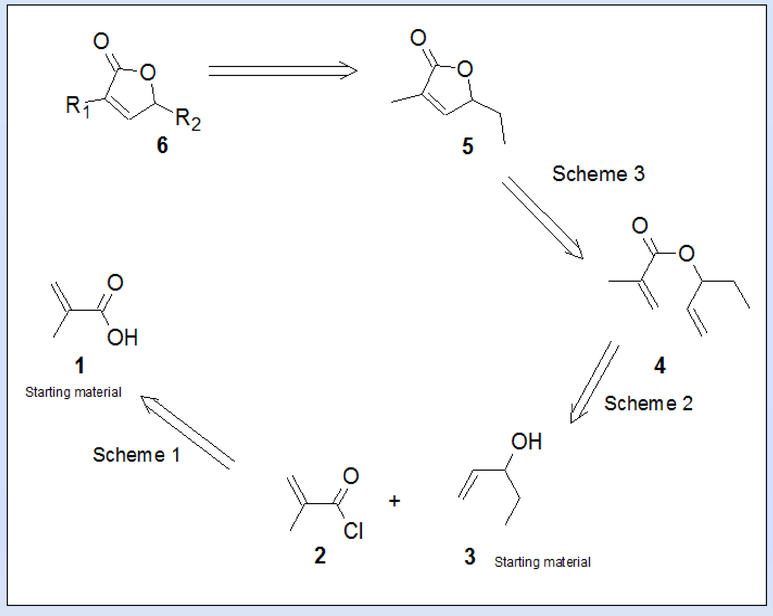
The proliferation of opportunistic mycoses by resident microbes is normally harmless but it may become virulent due to change in hosts' resistance or microbial environment. This can allow the microbes to become a health concern. *Candida* sp. are opportunistic pathogens and are responsible for 96% of infections, known as candidiasis (1). *Candida* infections can cause conditions that range from relatively mild muco-cutaneous infections to life-threatening candidemia and invasive candidiasis (2). In fact, it is the fourth most common cause of bloodstream infections in hospitalized patients (3). The treatment of candidiasis in AIDS and cancer patients is further complicated by the emergence of microbes that are resistant to existing triazole treatments (4). The most common species of yeast that causes candidiasis is *Candida albicans.*

The need for additional medicinal treatments has steered previous research toward the investigation of the coprophilous fungus, *Bombardioidea anartia.* This research led to the isolation of a group of metabolites called bombardolides, which exhibit activity against *Candida albicans*. Bombardolides are believed to be produced as secondary metabolites which inhibit the growth of competitors (5). The bombardolides are a group of butenolides, which are unsaturated cyclic esters or lactones.

In this research, efforts were made to develop an efficient pathway to synthesize butenolide derivatives that would exhibit activity against *Candida albicans*.

Retrosynthetic Analysis

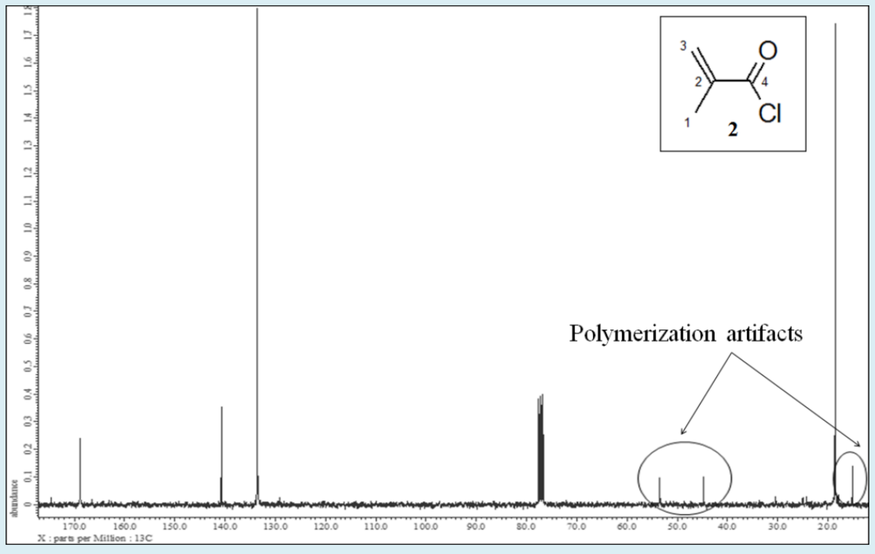
Close inspection of the butenolides suggests that ring-closing metathesis (RCM) could be used as an efficient tool for assembling the 5-membered lactones. Research conducted by M.Basetti *et al* suggests that cyclic butenolides can be prepared via RCM using Grubbs' first generation catalyst.



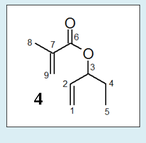
In the retrosynthetic analysis, the butenolide, 5-ethyl-3-methyl-2-(5H)-furanone (**5**) is synthesized from acrylate ester, pent-1-en-3-yl-2-methylprop-2-enoate (**4**). The ester is obtained by combining 1-penten-3-ol (**3**) and methacryloyl chloride (**2**) in the presence of Et_3N (**Scheme 2**). Methacryloyl chloride is obtained by chlorinating methacrylic acid (**1**) (**Scheme 1**).

Results and Discussion

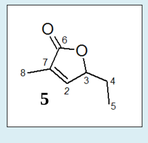
Compound **2** obtained from **Scheme 1** was characterized using ^{13}C- NMR spectroscopy. Additional peaks at 53.49, 44.76 and 15.38 ppm were due to unintended polymerization products of methacrylic acid (7).



The esterification in **Scheme 2** led to formation of compound **4**. Butenolide **5** was obtained from RCM of the ester in **Scheme 3.**



Ester 4 Scheme 2	
Carbon	ppm
1	116.6
2	129.1
3	77.6
4	27.3
5	9.6
6	166.8
7	136.6
8	18.4
9	125.3



Butenolide Scheme 3	
Carbon	ppm
2	131.0
3	68.2
4	26.3
5	8.5
6	172.2
7	132.5
8	18.8

The bioassay of compound **6** did not show activity against *Candida albicans*. However, it should be noted that active bombardolides have additional unsaturations at positions at 7 and 9.

Experimental

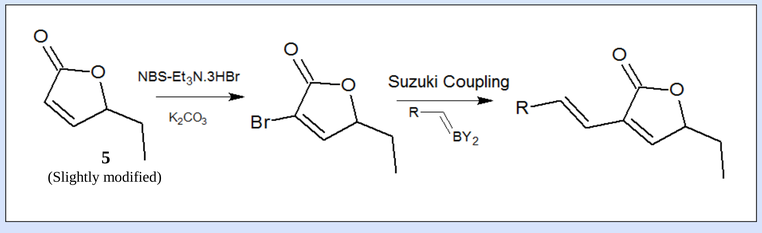
NMR Instrumentation: JEOL ECX 300 MHz equipped with an Oxford magnet and Delta software.
Scheme 1: Compounds **1** and **2** were refluxed in $SOCl_2$ for 1.5 hrs at 40-45^oC.
Scheme 2: Esterification of compounds **2** and **3** with Et_3N was carried out at 0^o C and stirred for 3 hrs under an inert atmosphere. The ester (**4**) was produced in 83.50% yield.
Scheme 3: RCM of compound **4** using Grubbs' first generation catalyst was carried out and stirred for 24 hours under an inert atmosphere. Purification was carried out using column chromatography (Si gel, hexanes/ethyl acetate). Butenolide **5** was isolated in 83.59% yield.
Bioassay: 20ug of sample was tested against *Candida albicans* using a disk diffusion assay.

Conclusion

From the results we conclude that it is possible to make the skeletal structure of the bombardolide-related derivatives by utilizing the outlined retrosynthetic pathway at an overall yield of about 70%. Even though the synthesized butenolide did not show activity against *Candida albicans,* it also did not have active functional groups in the R_1 and R_2 positions which are believed to be required for activity.

Future Work

Future plans are to synthesize butenolide derivatives with variable R_1 and R_2 groups using the skeletal structure of the prepared butenolide **5**.



Acknowledgement

1. Winona State University Undergraduate Student Research Grant and Travel Grant.
2. WSU Chemistry Department for use of facilities and resources.

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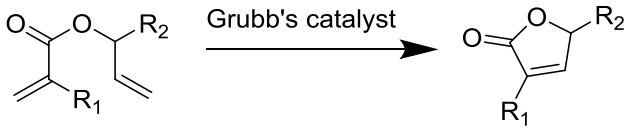
Investigation into the synthesis of bioactive butenolides

Abstract

Previous research done on coprophilous fungi has revealed the presence of secondary metabolites containing butenolides sub-structures. These butenolides show activity against the yeast *Candida albicans*. In efforts to further these investigations, the synthesis of butenolide analogs was undertaken. The starting materials, 2-methylacrylic acid and 1-penten-3-ol, were esterified in the presence of triethyl amine to form an ester, pent-1-en-3-yl-2-methylprop-2-enoate. The formation of ester was confirmed using ^{13}C- NMR. The ester was then subjected to ring-closing metathesis in the presence of Grubb's catalyst. The formation of the butenolide was also confirmed using ^{13}C-NMR. The product, 5-ethyl-3-methyl-2-(5H)-furanone obtained from this step has been purified using column chromatography. Attempts to derive the active butenolide and subsequent bioassay results will be presented.



Introduction

The proliferation of opportunistic mycoses by resident microbes is normally harmless but it may become virulent due to change in hosts' resistance or microbial environment. This can allow the microbes to become a health concern. *Candida* sp. are opportunistic pathogens and are responsible for 96% of infections, known as candidiasis (1). *Candida* infections can cause conditions that range from relatively mild muco-cutaneous infections to life-threatening candidemia and invasive candidiasis (2). In fact, it is the fourth most common cause of bloodstream infections in hospitalized patients (3). The treatment of candidiasis in AIDS and cancer patients is further complicated by the emergence of microbes that are resistant to existing triazole treatments (4). The most common species of yeast that causes candidiasis is *Candida albicans.*

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albicans. Bombardolides are believed to be produced as secondary metabolites which inhibit the growth of competitors (5). The bombardolides are a group of butenolides, which are unsaturated cyclic esters or lactones.

In this research, efforts were made to develop an efficient pathway to synthesize butenolide derivatives that would exhibit activity against *Candida albicans*.

Retrosynthetic Analysis

Close inspection of the butenolides suggests that ring-closing metathesis (RCM) could be used as an efficient tool for assembling the 5-membered lactones. Research conducted by M.Basetti *et al* indicates that cyclic butenolides can be prepared via RCM using Grubbs' first generation catalyst. Fig 1 shows the three step retrosynthetic pathway that was used to synthesize butenolide 5.

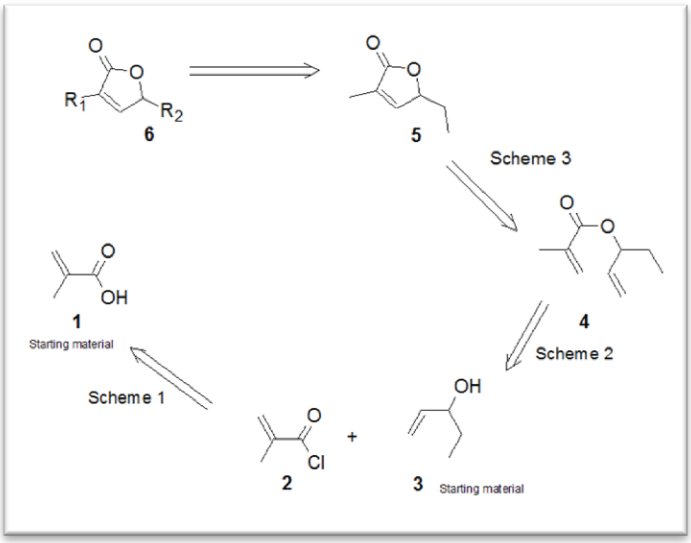


Figure 1: Retrosynthetic Analysis

In the retrosynthetic analysis, the butenolide, 5-ethyl-3-methyl-2-(5H)-furanone (**5**) is synthesized from acrylate ester, pent-1-en-3-yl-2-methylprop-2-enoate (**4**). The ester is obtained by combining 1-penten-3-ol (**3**) and methacryloyl chloride (**2**) in the presence of Et_3N (**Scheme 2**). Methacryloyl chloride is obtained by chlorinating methacrylic acid (**1**) (**Scheme 1**).

Experimental

Scheme 1: Compounds **1** and **2** were refluxed in $SOCl_2$ for 1.5 hrs at 40-45^oC.At the end of the reaction period a pale yellow reaction mixture containing methacryloyl chloride was obtained.

Scheme 2: Esterification of compounds **2** and **3** with Et_3N was carried out at 0^oC and stirred for 3 hrs in an inert atmosphere. For this 1-penten-3-ol and the reaction mixture from scheme 1 were taken in a ratio 1.2:1 by volume. The amount of tri-ethyl amine used was twice the mole of methacrylic acid used. The ester (**4**) was produced in 83.50% yield. A yellow solution of ester, pent-1-en-3-yl-2-methylprop-2-enoate was obtained. The solution was poured over cold water and extracted using dichloromethane. It was dried over Na_2SO_4. The solvent was removed under pressure and the residue was separated in a silica gel with hexane/diethyl ether as eluent.

Scheme 3: RCM of compound **4** using Grubbs' first generation catalyst was carried out and stirred for 24 hours in inert atmosphere. Several precautions were taken during this reaction. The reactants were used in very dilute concentration and catalyst was replenished in small quatities for a period of 3 hours. Purification carried out using column chromatography (Si gel). Butenolide **5** was isolated in 83.59% yield.

Bioassay: The Butenolide product tested in *Candida albicans* culture. Yeast broth solution (0.25ml in 50ml of agar) were introduced into autoclaved saboured tryptic agar (0.05g/ml). The agar was poured into plates and allowed to solidify. Tabs stained with 20ul of Filipin (1mg/ml) another containing 10mg/ml of the product were introduced into the plate. Filipin tab served as control. The plates were observed after a few days.

Results and Discussion

The compounds obtained from each scheme were characterized using ^{13}C- NMR spectroscopy. Fig 2 shows NMR spectrum for compound 2. The four peaks 1-4 in table 1 correspond to the four carbons in the acid chloride. Additional peaks at 53.49, 44.76 and 15.38 ppm did not belong to the acid chloride. The four peaks were attributed to unintended polymerization products of methacrylic acid at a temperature greater than 40^{o}C (7).

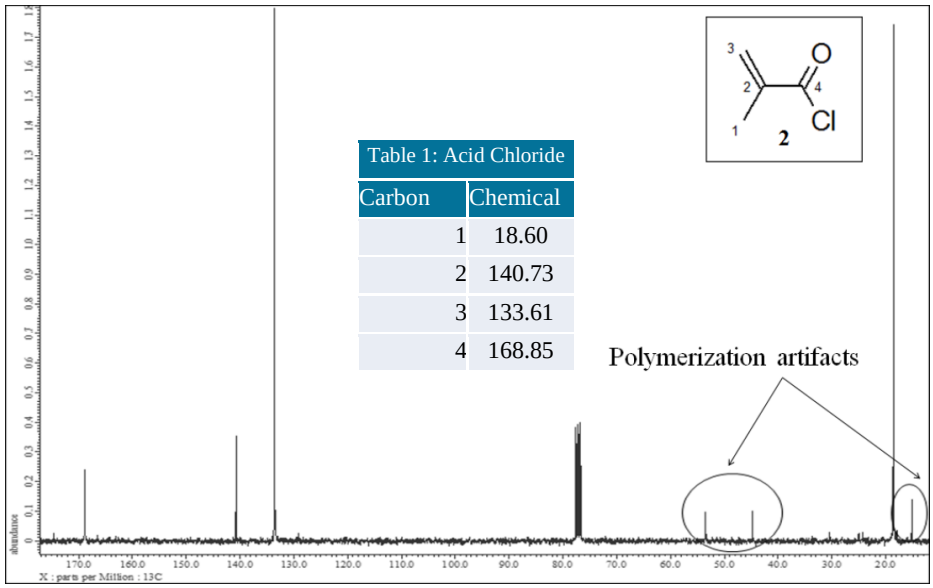
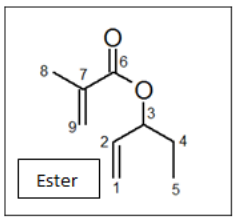


Figure 2: C-NMR of Acid Chloride

The esterification in **Scheme 2** led to formation of compound **4**. Butenolide **5** was obtained from RCM of ester in **Scheme 3.** The compounds were analyzed using ^{13}C- NMR. The NMR spectrums were compared to the spectrum of compounds synthesized in M.Basetti *et al.*



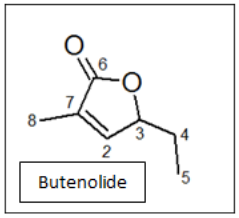


Table 2: Ester

Carbon	Chemical Shift(ppm)	
	Experimental	Literature
1	116.57	
2	129.06	
3	77.58	77.55
4	27.28	
5	9.58	
6	166.75	165.35
7	136.56	
8	125.25	
9	18.36	

Table 3: Butenolide

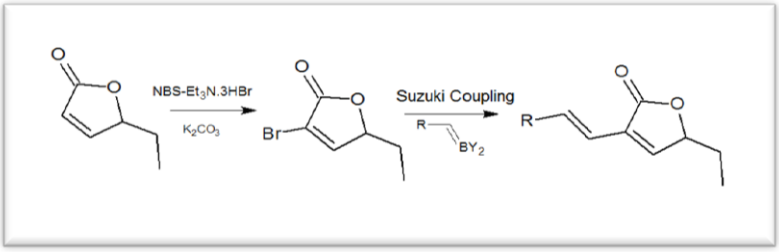
Carbon	Chemical Shift (ppm)	
	Experimental	Literature
2	130.91	
3	68.18	84.0
4	26.32	
5	8.49	
6	172.21	169.5
7	132.47	
8	18.81	

The C=O and C-O peaks of ester at 166.75 ppm and 77.58 ppm were very close to the peaks obtained from the literature article for a similar compound as shown in table 2. Likewise, comparison of spectrum for compound 5 showed peaks with similar chemical shift (table 3).

The plate bioassay did not show a zone of exhibition around butenolide. Hence, compound **5** did not have any activity against *Candida albicans*. However, it should be noted that active bombardolides have additional unsaturations at positions at 7 and 9 (5).

Conclusion

From the results, we conclude that it is possible to make the skeletal structure of the bombardolide-related derivatives by utilizing the outlined retrosynthetic pathway at an overall yield of about 70%. Even though the synthesized butenolide did not show activity against *Candida albicans*, it also did not have active functional groups in R_1 and R_2 positions which are believed to be required for activity. Future plans are to synthesize butenolide derivatives with variable R_1 and R_2 groups using the skeletal structure of the prepared butenolide **5**.



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